**ITEM 1:**

## SCIENTIFIC ABSTRACT

This proposal is a Phase I/II study to examine the efficacy of, and toxicity associated with, adoptive immunotherapy using simultaneously-*ex vivo*-expanded cytotoxic T-lymphocytes (CTL) for prophylaxis against Epstein-Barr virus (EBV) and cytomegalovirus (CMV) complications in recipients of CD34 selected/T-cell depleted stem cell transplants (SCT).

EBV-induced lymphoproliferative disease and CMV infection are major causes of morbidity and mortality in individuals with compromised cellular immunity. Although anti-viral pharmacological agents exist, severe side effects such as myelosuppression often limit the application of such medication. Infusion of *ex vivo* expanded, virus-specific CTL has been proven to be safe and efficacious for the prophylaxis and treatment of EBV and CMV complications. While EBV-specific CTL can be readily and reliably produced with EBV-immortalized B-lymphoblastoid cell lines (BLCL) as stimulators, current protocols for CMV-specific CTL, which use CMV-infected fibroblasts as stimulators, often have shortfalls such as alloreactivity, a long period of time for culture, and potential exposure to human blood-born infectious agents.

Our laboratory has developed a novel system to generate EBV/CMV-bi-specific CTL culture by co-culturing PBMC with autologous BLCL expressing a CMV protein pp65 (BLCLpp65). pp65, an immunodominant CMV antigen, is transduced into BLCL by recombinant retrovirus MSCVpp65. While low in alloreactivity, BLCLpp65-stimulated CTL are cytolytic to autologous cells infected with EBV or CMV, and this cytotoxicity is mediated by polyclonal, CD8+, MHC Class I-restricted T-cells. Further experiments revealed that the EBV-specific cytotoxicity in the CTL primed with BLCLpp65 is similar in spectrum of antigen recognition and comparable in intensity of specific killing to those stimulated with BLCL. These data indicated that BLCLpp65 could substitute for BLCL as antigen presenting cells in adoptive immunotherapy against EBV-LPD, with the benefit of providing protection against CMV reactivation. Significantly, preparation of EBV- and CMV-specific CTL in the same culture would also simplify production and administration of the therapeutic/prophylactic CTL.

EBV/CMV-specific CTL used for infusion will be generated from peripheral blood mononuclear cells (PBMC) of EBV/CMV-seropositive donors over a 21-28 day period by weekly stimulation with autologous BLCLpp65. Qualified CTL will be administered to consenting recipients of CD34 selected/T-cell depleted SCT at 40, 60 and 80 days post-transplant. Quality control tests for the CTL preparation and production intermediates include specific cytotoxicity, sterility, endotoxin, replication-competent retroviruses (RCR) and other adventitious viruses. Efficacy of the immunotherapy will be evaluated by criteria of molecular virology and immunological reconstitution. The former will be accomplished by measuring blood levels of CMV antigens and EBV viral DNA, while the latter will be achieved by assessing virus-specific CTL frequency. Post-infusion patients will also be tested for RCR at 3, 6 and 12 month intervals post-transplant to ensure bio-safety. Results will be released to patients and data will be communicated in peer-reviewed journals.